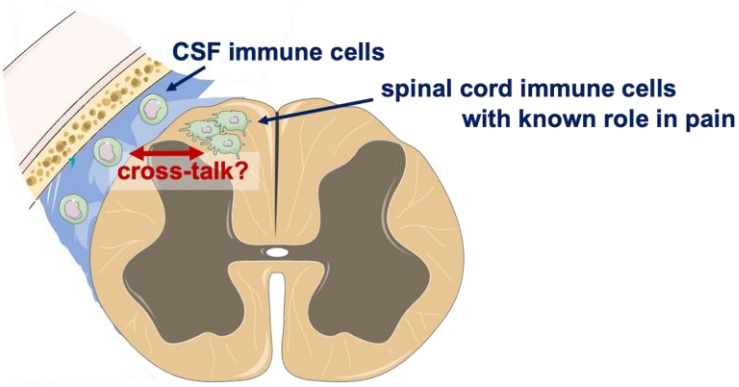
Cerebrospinal fluid- a window into how our central nervous system processes pain?

Background & Rationale: This study aimed to find out whether we can use cerebrospinal fluid (which bathes our brain and spinal cord) to better understand chronic pain.



Research suggests that immune cells in the spinal cord play an important role in maintaining painful conditions, especially when they are caused by direct damage to nerves. Yet, for obvious reasons, studying these cells directly is impossible in people.

Our team set out to investigate whether we can instead assess the state of these cells by looking at the cerebrospinal fluid (CSF). It is known that substances released in spinal cord can leak into the fluid. Also, there are immune cells swimming about in the CSF – just across from spinal cord immune cells, and it is possible that these cell types are talking to each other.

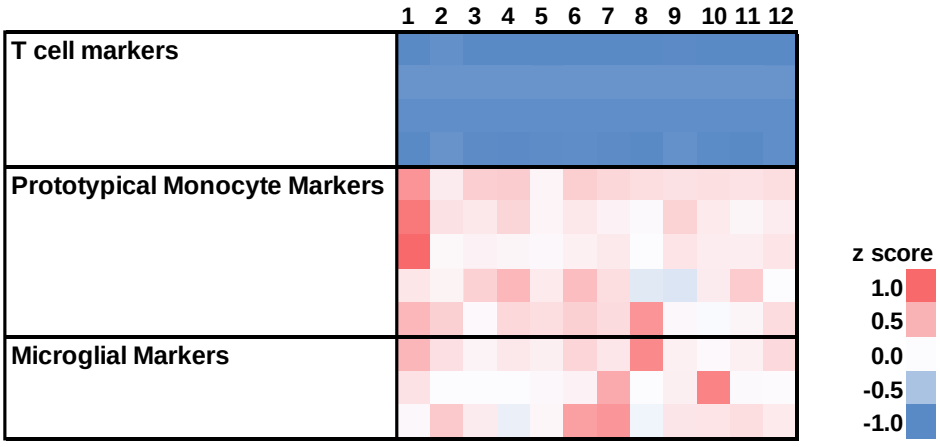
To test whether this is the case, we analysed CSF generously donated by individuals who were undergoing neuromodulation surgery to treat their pain. We looked at the substances in the fluid, as well as the immune cells, with a technique called RNA sequencing.

What did we find? The COVID19 pandemic naturally presented some challenges to our recruitment efforts, but we managed to collect enough data to get some answers to the questions we set in our proposal. These were:

1) *Can we use CSF as a way to measure spinal cord immune cell activation?*

In the past, research has observed that certain proteins are more prevalent in the CSF of chronic pain patients. These are proteins that could be released from the immune cells in the spinal cord or from those in the CSF itself.

Our project showed that certain immune cells in CSF, known as monocytes, are capable of making substances also produced by spinal cord immune cells, known as microglia. This includes substances usually assumed to be “microglial-specific”:



CSF monocytes from individuals living with chronic pain, analysed for their expression of certain maker genes; as expected, they do not express marker genes for T cells (another immune cell type), but express genes typically found in monocytes and microglia. The redder a

square, the more expression of a given marker. The bluer a square, the less of a certain marker is present.
Overall, our results suggest that whatever we measure in CSF does not necessarily reflect spinal cord immune cell activation. However, CSF immune cell composition and expression still opens a window into immune dysregulation in pain more generally.

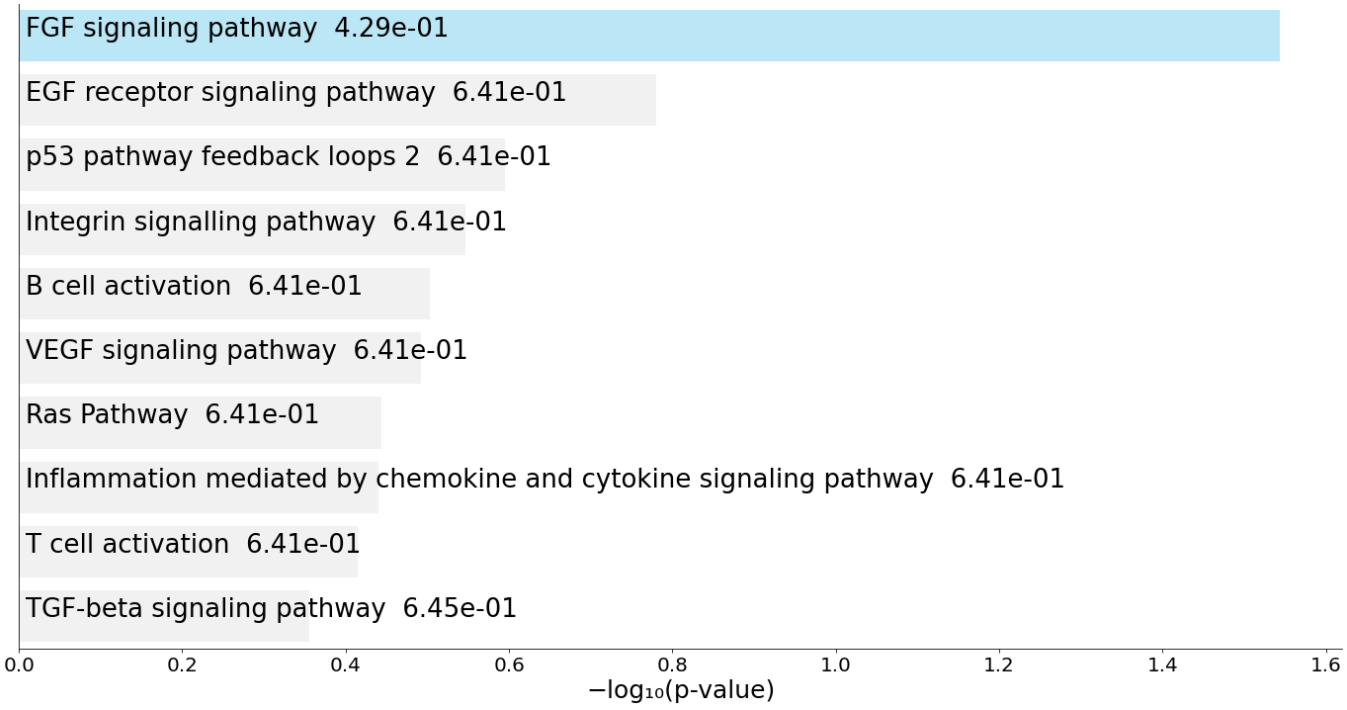
2) *Can we detect any differences in CSF immune profiles that are related to the kind of pain someone experiences?*

Our sample collection suggests that individuals with quite divergent chronic pain origins seemingly have very similar immune cell numbers and populations in their CSF.

However, when you examine these immune cells more closely, looking at what genes they express, differences start to emerge. This is especially true for immune cells known as monocytes. In our samples, monocytes looked different in individuals who had pain as a result of a back injury and/or surgery compared to those who had more widespread pain.

There were a number of proteins which stood out, including cytokines and markers of monocyte subsets. Moreover, certain growth factor pathways were enriched, e.g. the FGF signalling pathway. We will expand on these in a manuscript that we are preparing.

Signalling pathways that were enriched in monocytes from one pain patient cohort compared to another:



3) *Can we use CSF as a way to predict whether someone will benefit from the implantation of high frequency stimulators to treat pain?*

This last question is one where our answer has the most uncertainty attached to it. We saw some interesting differences in CSF content before and after spinal cord stimulation. However, these differences were observed ‘after the fact’ at group level, that is once we had divided samples into pre- and post-stimulation.

It is therefore unclear whether the differences would be strong enough to predict treatment success, if all we had are counts of a certain immune cell population in a single individual. To answer this question fully, we require additional, larger scale-studies.
What will we do next? We are working on a manuscript to publish our results, specifically the data on which spinal cord immune cell populations are present in the different pain conditions and how they differ from each other.

Our data will be presented in a way that will allow them to be fully integrated into future studies, i.e. other scientists will be able to combine their findings with our results. This is very important, as it will speed up replication and allow direct comparisons to other study cohorts.

Finally, a very big ***thank you*** to the individuals who generously donated CSF for this study. Without them, none of this work would have been possible.